

Amendments to the Specification

Following the abstract, please insert the uploaded Sequence Listing filed herewith.

Please replace paragraph [0065] of the published application with the following amended paragraph:

[0065] FIG. 11 shows EV1 binding inhibited in the presence of anti- $\alpha_2\beta_1$. Binding of [^{35}S]-methionine labeled EV1 to ovarian cancer cell lines in the presence and absence of either anti- $\alpha_2\beta_1$ or anti-DAF MAbs. Levels of [^{35}S]-methionine labeled virus bound was determined by liquid scintillation counting on a 1450 ~~Mierobeta~~ MICROBETA® TRILUX (Wallac, Finland).

Please replace paragraph [0070] of the published application with the following amended paragraph:

[0070] FIG. 16 shows binding of [^{35}S]-methionine labeled EV1 to SkMel28 melanoma cells in the presence and absence of either anti- $\alpha_2\beta_1$ or anti-DAF MAbs. Levels of [^{35}S]-methionine labeled virus bound was determined by liquid scintillation counting on a 1450 ~~Mierobeta~~ MICROBETA® TRILUX (Wallac, Finland). $\alpha_2\beta_1$ blockade resulted in significant inhibition of EV1 binding. Results are expressed as the mean of triplicate samples \pm standard error.

Please replace paragraph [0103] of the published application with the following amended paragraph:

[0103] Enteroviral receptor surface expression on cancer cells was analysed by flow cytometry. Dispersed cells (1×10^6) were incubated for 20 minutes on ice with the appropriate MAb (5 $\mu\text{g}/\text{ml}$ diluted in PBS) for 20 minutes. Cells were washed with PBS and pelleted by centrifugation before resuspension in 100 μl of 1:50 dilution of R-phycoerythrin-conjugated F(ab')₂ fragment of goat anti-mouse immunoglobulin (Dako, A/S, Denmark). Cells were again incubated on ice for 20 minutes, washed, pelleted and resuspended in PBS prior to flow

cytometric analysis. Cell surface receptor expression was analysed using a FACStar™ Analyser (Becton Dickenson, Sydney, Australia).

Please replace paragraph [0110] of the published application with the following amended paragraph:

[0110] Six-well tissue culture plates containing confluent monolayers of DOV13 cells were inoculated with 500 μ l EV1 (multiplicity of infection [moi]= 10^5 TCID₅₀/ml) for 1 hour at 37°C. Unbound virus was removed by washing three times with methionine/cysteine free DMEM (ICN Biomedical, Ohio, USA) and cell monolayers were incubated in 1.3 ml of this media for a further 2 hours at 37° C. before addition of 300 μ Ci of [³⁵S]-methionine translabel (ICN Biomedical, Ohio, USA). Infected monolayers were incubated overnight at 37° C. in a 5% CO₂ environment. Following three freeze/thaw cycles viral lysates were purified in a 5-30% sucrose gradient by velocity centrifugation for 95 minutes at 36,000 rpm in a Beckman XL-90 ultracentrifuge (SW41ti Rotor). Fractions were collected from the bottom of each tube and monitored by liquid scintillation counting (Wallac 1450' Micropbeta MICROBETA® TRILUX, Finland) to locate 160S viral peak fraction used in viral binding assays.

Please replace paragraph [0113] of the published application with the following amended paragraph:

Approximately 1x10⁶ cells resuspended in 800 μ l of RPMI containing 1% bovine serum albumin (BSA) were incubated in the presence of 20 μ g/ml of MAb (anti- $\alpha_2\beta_1$ or anti-DAF diluted in PBS) for 1 hour at 4° C[.] followed by the addition of 300 μ l (1x10⁶) of [³⁵S]-methionine labeled 160S EV1. After incubation at 4° C[.] for 2 hours cells were washed four times with serum free media and cell pellets dissolved in 200 μ l 0.2M NaOH-1% SDS before the level of [³⁵S]-methionine labeled virus bound was determined by liquid scintillation counting from triplicate samples. (Wallac 1450 Micropbeta MICROBETA® TRILUX, Finland). Results were expressed as means±SE.

Please replace paragraph [0115] of the published application with the following amended paragraph:

[0115] $[^{35}\text{S}]$ -methionine labeled viral fractions were analysed by polyacrylamide gel electrophoresis (PAGE) and visualised by autoradiography. $[^{35}\text{S}]$ -methionine labeled 160S EV1 fractions were incubated with sample reducing buffer (250 mM TRIS, 0.2 g w/v SDS, 20% v/v glycerol, 10% v/v 2-mercaptoethanol and 0.01% w/v bromophenol blue, pH 6.8) for 10 minutes at 95° C[.] denaturing the virion. Denatured 160S vial peak fractions were then separated on a 15% Tris-HCl precast gel (BIORAD Ready-Gel Bio-Rad READY GEL®, CA, USA) in conjunction with a Benchmark prestained midrange protein ladder (GIBCO, USA) at 180 V for 45 minutes. Visualisation of the major structural proteins and analysis of viral purity was by autoradiography on Hyperfilm MP (Amersham International, England) after 96 hour exposure.

Please replace paragraph [0117] of the published application with the following amended paragraph:

[0117] Cell suspensions of human peripheral blood lymphocytes, OVHS-1 and DOV-13 cells were challenged with EV1 (moi=1.0 TCID_{50} TCID_{50} /cell) and incubated for 24 h at 37° C. Levels of cell cytolysis were calculated as a function of release of LDH (a stable cytosolic enzyme that is released upon cell lysis), assessed by using a Cytotoxic CYTOTOX 96® kit (Promega Corp. Maddison, Wis. USA) as per the manufacturers instructions.

Please replace paragraph [0119] of the published application with the following amended paragraph:

[0119] DOV-13 cells were seeded in a 24-well plate at 500 or 5000 cells per well in 1 ml of RPMI 1640 containing 5% FCS onto a semi-solid 0.5% agarose layer. Cells were incubated for 48 h at 37°C in a 5% CO_2 atmosphere to allow spheroids to form, before the addition of EV1 (10^5 TCID_{50}).

Please replace paragraph [0123] of the published application with the following amended paragraph:

[0123] Sera from infected mice were analyzed for viremia using real-time quantitative RT-PCR. Briefly, viral RNA was extracted from 10 μ l of serum using a QIAamp[®] Viral RNA mini kit (Qiagen, Clifton Hill, Victoria, Australia) and eluted in a final volume of 40 μ l according to manufacturer's instructions. Primers and probe for determination of EV1 viral RNA levels were designed using the Primer Express PRIMER EXPRESS[™] 1.5 software (Applied Biosystems, Foster City, Calif., USA) and were based on the previously published EV1 sequence (Genbank accession number AF029859); forward primer ($5'$ -CAAGACAGGGACCAAAGAGGAT- $3'$) (SEQ ID NO: 1), reverse primer ($5'$ -CCACTCGCCTGGTTGTAATCA- $3'$) (SEQ ID NO: 2) and 6-FAM-labeled MGB-probe ($5'$ -CCAATAGCTTCAACAATT- $3'$) (SEQ ID NO: 3). One-step RT-PCR was performed using Platinum[®] PLATINUM[®] Quantitative RT-PCR ThermoScript[®] THERMOSCRIPT[™] One-Step System on an ABI 7000 sequence detector. For generation of the standard curve, 10-fold dilutions of EV1 viral stock (1×10^6 TCID₅₀/ml) was amplified with optimized concentration of primers and probe. In a volume of 25 μ l, the reaction mixture comprised[[.]]; 1x ThermoScript[®] THERMOSCRIPT[™] reaction mix, 500 nM forward, 900 nM reverse primer, 250 nM probe, 500 nM ROX, 0.5 μ l ThermoScript[®] THERMOSCRIPT[™] Plus/Platinum Taq Mix and 5 μ l extracted RNA. Thermal cycling conditions were subjected to 30 min at 60° C[[.]], followed by 5 min at 95° C[[.]] and then 40 cycles of 15 s at 95° C[[.]] and 1 min at 60° C.